

CLAIM AMENDMENTS

1. (currently amended) A method of crosslinking a first and a second moiety comprising the steps of:

providing a first moiety having a covalently attached~~ing~~a first metal ligand to the first moiety, the first moiety comprising and at least one phenolic group or phenolic derivative thereof;

providing a second moiety comprising fluorescein or fluorescein-containing derivative thereof having a covalently attached~~ing~~a second metal ligand to the second moiety and, the second moiety comprising at least one phenolic group or phenolic derivative;

adding a coordinating metal ion to a reaction mixture containing the first moiety and the second moiety, thereby forming a metal coordination complex between the metal ion and each of the first moiety and the second metal ligands, moiety; wherein the coordination complex provides said phenolic groups and/or phenolic derivatives thereof of the first and second moieties at a distance sufficient for crosslinking there between; and

crosslinking the first and second moieties by exposing the metal coordination complex to an oxidizing agent, to form a thereby covalently crosslinking the first moiety to the second moiety by between the phenolic groups and/or the phenolic derivatives thereof attached to each of the first and second moieties.

2. (currently amended) The method of claim 1, wherein the metal coordination complex activates the oxidizing agent.

3. (original) The method of claim 1, wherein the oxidizing agent is activated by a metalloenzyme.

4. (original) The method of claim 3, wherein the metalloenzyme is selected from the group consisting of a peroxidase, a tyrosinase, a laccase, and a catechol oxidase.

5. (currently amended) The method of claim 4, wherein the peroxidase is horseradish peroxidase.

6. (original) The method of claim 1, wherein the oxidizing agent is generated electrochemically at a surface of an electrode.

7. (original) The method of claim 1, wherein the phenolic groups or phenolic derivatives are each selected from the group consisting of tyrosine, dihydroxyphenylalanine, and polyphenolic compounds.

8. (currently amended) The method of claim 1, wherein the second moiety comprises at least one phenolic group positioned such that in the metal coordination complex the phenolic group is located between 1 and 100 angstroms from the metal ion.

9. (original) The method of claim 1, wherein the first moiety comprises at least one phenolic group positioned such that in the coordination complex the phenolic group is located between 1 and 100 angstroms from the metal ion.

10. (original) The method of claim 9, wherein the phenolic group is a tyrosine residue located on the first moiety.

11. (currently amended) The method of claim 9, wherein the phenolic group is a tyrosine residue located within the metal ligand on the first moiety and positioned such that in the metal coordination complex the tyrosine is located between the metal ion and the first polymer.

12. (original) The method of claim 1, wherein the covalent crosslink is a substituted phenolic adduct.

13. (original) The method of claim 1, wherein the covalent crosslink is formed by a chemical bond between the phenolic group or phenolic derivative on the first moiety and the phenolic group or phenolic derivative on the second moiety.

14. (original) The method of claim 1, wherein the covalent crosslink is dityrosine and isomers thereof.

15-19. (canceled).

20. (original) The method of claim 1, wherein at least one of the first and second moieties is a synthetic polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, polyesters, and polyethylene glycol and polypropylene glycol block copolymers.

21. (previously presented) The method of claim 1, wherein the first moiety is a polymer.

22-25. (canceled).

26. (original) The method of claim 1, wherein one of the first and second moieties is attached to a solid surface.

27. (original) The method of claim 26, wherein the solid surface is selected from the group consisting of a polymer, a metal, a ceramic, a composite, a biopolymer, a bioceramic, and a colloidal particle.

28. (original) The method of claim 27, wherein the solid surface is a metal and is coated with a polymer.

29. (original) The method of claim 27, wherein the solid surface is a colloidal particle and the colloidal particle is composed of a material selected from the group consisting of gold, silver, silica, semiconductors, fluorescent semiconductors, polystyrene, polymeric micelles, dendrimers, liposomes, and viruses.

30. (original) The method of claim 27, wherein the surface is a colloidal gold particle and the colloidal particle has a diameter of from 1 nm to 100 μ M.

31. (original) The method of claim 1, wherein both the first and second moieties are attached to a solid surface.

32. (original) The method of claim 31, wherein the first and second moieties are attached to different solid surfaces and the crosslinking is used to adhere two solids.

33. (original) The method of claim 32, wherein the two solid surfaces are biological tissues.

34. (original) The method of claim 32, wherein each of the solid surfaces are independently selected from the group consisting of polymers, metals, ceramics, composites, biopolymers, bioceramics, colloidal particles, and combinations thereof.

35. (original) The method of claim 34, wherein at least one of the solid surfaces is a colloidal particle, and the colloidal particle is composed of a material selected from the group consisting of gold, silver, silica, semiconductors, fluorescent semiconductors, polystyrene, polymeric micelles, dendrimers, liposomes, and viruses.

36. (original) The method of claim 35, wherein the colloidal particle has a diameter from 1 nm to 100 μ M.

37. (original) The method of claim 1, wherein at least one of the first moiety and the second moiety is biodegradable.

38. (original) The method of claim 1, wherein at least one of the first moiety and the second moiety contains a therapeutic agent.

39. (original) The method of claim 38, wherein the therapeutic agent is a protein.

40. (original) The method of claim 1, wherein the first moiety is an HY-tag.

41. (original) The method of claim 40, wherein the first moiety contains plurality of tyrosine residues interdispersed through out the HY-tag.

42. (original) The method of claim 40, wherein the HY-tag comprises a plurality of histidine residues.

43. (original) The method of claim 1, wherein the metal ion is selected from the group consisting of nickel, copper, zinc, and cobalt, gadolinium, iron, osmium, palladium, rhodium, ruthenium, samarium, selenium, silver, strontium, tantalum, thulium, tin, tungsten, vanadium, yttrium, and zinc.

44. (original) The method of claim 43, wherein more than one metal ion is present in the coordination complex.

45. (withdrawn) A method of crosslinking a first and a second protein comprising the steps of:

- attaching a first HY-tag to a first protein;
- attaching a second HY-tag to a second protein;
- creating a coordination complex between the first protein, the second protein, and a metal ion; and
- crosslinking the first and second proteins at or adjacent to the HY-tags by exposing the coordination complex to an oxidizing agent to form a covalent crosslink.

46. (withdrawn) The method of claim 45, wherein the coordination complex is catalytic or redox active.

47. (withdrawn) The method of claim 45, wherein the oxidizing agent is a mild oxidizing agent and crosslinking occurs without significant non-specific crosslinking.

48. (withdrawn) The method of claim 47, wherein the oxidizing agent is provided as Na_2SO_3 .

49. (withdrawn) The method of claim 45, wherein the oxidizing agent is a peroxide.
50. (withdrawn) The method of claim 49, wherein the peroxide is H₂O₂.
51. (withdrawn) The method of claim 47, wherein the oxidizing agent is O₂.
52. (withdrawn) The method of claim 45, wherein the first and second HY-tags each comprise a tyrosine residue located between a plurality of histidine residues and the protein.
53. (withdrawn) The method of claim 53, wherein the covalent crosslink is a substituted dityrosine.
54. (withdrawn) A method of conjugating a protein with a synthetic polymer comprising the steps of:
attaching a metal-binding peptide to a selected protein;
attaching a second ligand to a selected polymer;
forming a coordination complex between the protein, the polymer, and a metal ion; and
crosslinking the protein and polymer by exposing the coordination complex to an oxidizing agent.
55. (withdrawn) The method of claim 54, wherein the coordination complex is redox active.
56. (withdrawn) The method of claim 54, wherein the polymer is a synthetic polymer.
57. (withdrawn) The method of claim 54, wherein the polymer is polyethylene glycol.
58. (withdrawn) The method of claim 54, wherein the metal ion is selected from the group consisting of nickel, copper, zinc, and cobalt.

59. (withdrawn) The method of claim 54, wherein the oxidizing agent is Na_2SO_3 or O_2 .
60. (withdrawn) The method of claim 54, wherein the oxidizing agent is a peroxide.
61. (withdrawn) The method of claim 58, wherein the oxidizing agent is H_2O_2 .
62. (withdrawn) The method of claim 54, wherein the oxidizing agent is a mild oxidizing agent and crosslinking occurs without significant non-specific crosslinking.
63. (withdrawn) The method of claim 54, wherein one of the metal-binding peptide is a HY-tag and the second ligand is a synthetic chelator.
64. (withdrawn) A method of immobilizing a protein on a polymer surface under conditions that preserve protein structure and activity, comprising the steps of:
modifying a polymeric surface such that the polymeric surface comprises a synthetic ligand;
attaching a metal-binding peptide to a protein;
forming a coordination complex between the polymeric surface, the protein, and a metal ion; and
immobilizing the protein to the polymeric surface by exposing the coordination complex to an oxidizing agent, thus causing the crosslinking of the polymer and the protein.
65. (withdrawn) The method of claim 64, wherein the polymer surface is further comprised of a synthetic polymer.
66. (withdrawn) The method of claim 64, wherein the polymer is polyethylene glycol.
67. (withdrawn) The method of claim 64, wherein the metal ion is selected from the group consisting of nickel, copper, zinc, and cobalt, gadolinium, iron, osmium, palladium, rhodium, ruthenium, samarium, selenium, silver, strontium, tantalum, thulium, tin, tungsten, vanadium, yttrium, and zinc.

68. (withdrawn) The method of claim 64, wherein the oxidizing agent is a mild oxidating agent.

69. (withdrawn) The method of claim 64, wherein the metal-binding peptide is a HY-tag.

70. (withdrawn) A crosslinked polymer material produced by crosslinking a polymer having a synthetically-placed chelator in a mixture comprising the polymer, a metal, and an oxidant.

71. (withdrawn) The crosslinked polymer material of claim 70, wherein the polymer is a protein.

72. (withdrawn) The crosslinked polymer material of claim 71, wherein the synthetically-placed chelator is a HY-tag.

73. (withdrawn) The crosslinked polymer material of claim 72, wherein the protein comprises two HY-tags and the protein is polymerized by the crosslinking.

74. (withdrawn) The crosslinked polymer material of claim 71, wherein the mixture further comprises a synthetic polymer comprising a chelator, and wherein the synthetic polymer is crosslinked to the protein.

75. (withdrawn) The crosslinked polymer material of claim 70, wherein the mixture comprises a plurality of identical polymers each having the synthetically-placed chelator.

76. (withdrawn) The crosslinked polymer material of claim 70, wherein the mixture comprises a second polymer having a synthetically-placed chelator, the second polymer being different from the first polymer and crosslinked thereto.

77. (withdrawn) The crosslinked polymer material of claim 76, wherein the polymer is a protein and the second polymer is a synthetic polymer.

78. (withdrawn) The crosslinked polymer material of claim 77, wherein the second polymer is a polymeric surface.

79. (withdrawn) The crosslinked polymer material of claim 70 wherein the oxidant is a mild oxidant.